Subunits of the Yeast SWI/SNF Complex Are Members of the Actin-related Protein (ARP) Family*

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Craig L. Peterson‡§, Yingming Zhao‡, and Brian T. Chait¶

From the ‡Program in Molecular Medicine and Department of Biochemistry and Molecular Biology, University of Massachusetts Medical Center, Worcester, Massachusetts 01605 and the ¶Laboratory for Mass Spectrometry and Gaseous Ion Chemistry, The Rockefeller University, New York, New York 10021

The yeast SWI/SNF chromatin remodeling complex is comprised of 11 tightly associated polypeptides (SW1, SWI2, SWI3, SNF5, SNF6, SNF11, SWP82, SWP73, SWP59, SWP61, and SWP29). We have used matrix-assisted laser desorption ionization time-of-flight mass spectrometry to identify the genes that encode the SWP59 and SWP61 subunits. Surprisingly, we find that SWP59 and SWP61 are encoded by the ARP9 and ARP7 genes, respectively, which encode members of the actin-related protein (ARP) family. Sequence analyses have shown that ARP9 and ARP7 are 24–26% identical (48–51% similar) to yeast actin and that they are likely to maintain the overall actin fold. Deletion of either the ARP9 or ARP7 gene causes typical swi/snf phenotypes, including growth defects on media containing galactose, glycerol, or sucrose as sole carbon sources. ARP9 and ARP7 are also required for expression of an HO-lacZ fusion gene and for full transcriptional enhancement by the GAL4 activator. The identification of two ARP family members as crucial subunits of the SWI/SNF complex suggests that the complex may contain a total of three different ATPase subunits; furthermore, the similarity of ARP7 and ARP9 to the HSP and HSC family of ATPases suggests the possibility that chromatin remodeling by SWI/SNF may involve chaperone-like activities.

The Actin-related protein (ARP) family is a branch of the larger actin superfamily of proteins that includes conventional actins, heat shock protein 70 (Hsp70), sugar kinases, glycerol kinase, and other ATP-binding proteins from prokaryotic and eukaryotic sources. Surprisingly, we find that SWP59 and SWP61 are encoded by the ARP9 and ARP7 genes, respectively, which encode members of the ARP family. Sequence analyses have shown that ARP9 and ARP7 are 24–26% identical (48–51% similar) to yeast actin and that they are likely to maintain the overall actin fold. Deletion of either the ARP9 or ARP7 gene causes typical swi/snf phenotypes, including growth defects on media containing galactose, glycerol, or sucrose as sole carbon sources. ARP9 and ARP7 are also required for expression of an HO-lacZ fusion gene and for full transcriptional enhancement by the GAL4 activator. The identification of two ARP family members as crucial subunits of the SWI/SNF complex suggests that the complex may contain a total of three different ATPase subunits; furthermore, the similarity of ARP7 and ARP9 to the HSP and HSC family of ATPases suggests the possibility that chromatin remodeling by SWI/SNF may involve chaperone-like activities.

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‡ Scholar of the Leukemia Society of America. To whom correspondence should be addressed: UMMC, 373 Plantation St., Biotech 2, Suite 301, Worcester, MA 01605. Tel.: 508-556-5858; Fax: 508-556-4288; E-mail: craig.peterson@ummed.edu.

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results have major implications for the role of ATP in SWI/SNF-dependent chromatin remodeling.

**EXPERIMENTAL PROCEDURES**

**MALDI-TOF Mass Spectrometry Analysis**—The SWI/SNF complex was purified from a 20-liter culture of yeast strain CY396 as described previously (16). The final Superose 6 pool was precipitated with 15% final concentration of trichloroacetic acid, the protein pellet was washed with −20 °C acetone, dried, and resuspended in 20 μl of SDS sample buffer. The protein sample was separated by 10% SDS-PAGE, and SWI/SNF subunits were visualized by copper staining (Bio-Rad). The bands of interest were excised, subjected to in-gel digestion with trypsin, and the resulting peptide mixtures extracted as described previously (17). Peptide mixtures were analyzed with a MALDI-TOF mass spectrometry using delayed ion extraction and ion mirror (Voyager-DE STR, Perseptive Biosystems, Inc., Framingham, MA). The accurately measured masses of the tryptic peptides were used to search for protein candidates in SWISS-PROT protein sequence data base with the program “ProFound” (18) (http://prowl/PROWL/prot-id-main.html).

**Strains and Media**—All strains are congenic to S288C and are isogenic derivatives of strain yPH274 (19). Strains isogenic to CY114 contain a chromosomal HO-lacZ fusion gene integrated at the HO locus. Cultures were grown at 30 °C in YEP medium (2% yeast extract, 1% bacto-peptone) containing 2% final concentration of either glucose, galactose, or sucrose. Media containing galactose or sucrose also contained 1 μg/ml of antimycin. Minimal medium contained 6.7 g/liter yeast nitrogen base without amino acids (Difco), supplemented with amino acids as described (20).

**ARP7 and ARP9 Deletion Alleles**—Precise replacements of the ARP7 or ARP9 coding regions with the HIS3 gene product (GenBank™ accession number 809599) by PCR approach. 50-Mer oligonucleotides (Genosys) contained 61 base pairs of homology to either the 5′ or 3′ ends of the ARP7 or ARP9 coding region and 19 base pairs of homology to either the 5′ or 3′ ends of the HIS3 locus. PCR primers had the following sequences: ARP7 5′-CTGAGTATGGCTATTTAGCAAABCGCGGAGAATTACTATTATAAACAGGATGTGCTAACAGGGCTCCCTCTAGTACCTC-3′; ARP7 3′-ATGTTTGGCGCTTACGGCGGCGCCCTCTTCAACAGAA-3′ (GenBank™ accession number 809599); ARP7 5′ (5′-3′), GGTCGAGATCTCATCTCCTTCTAGCCGCCTACAATCCCTTTTGGATG-3′ (5′-3′), AATTAGAAAATGTTACACCGGAGAATTACTATTATAAACAGGATGTGCTAACAGGGCTCCCTCTAGTACCTC-3′; ARP9 5′ (5′-3′), ATATTTGCGCCTTACCGCGCGCCTCGTTCAGAATG-3′ (5′-3′), AATTAGAAAATGTTACACCGGAGAATTACTATTATAAACAGGATGTGCTAACAGGGCTCCCTCTAGTACCTC-3′; ARP9 3′ (5′-3′), AAACAAAAAGCAACATATTCCACACGGGATTTTGGATACCGGAACTACTATAGCATTGAAGGGTCTCCCTCTATGAC-3′; ARP9 3′ (5′-3′), AAACAAAAAGCAACATATTCCACACGGGATTTTGGATACCGGAACTACTATAGCATTGAAGGGTCTCCCTCTATGAC-3′; ARP9 3′ (5′-3′), AAACAAAAAGCAACATATTCCACACGGGATTTTGGATACCGGAACTACTATAGCATTGAAGGGTCTCCCTCTATGAC-3′.

Each pair of primers was used to amplify the HIS3 locus from plasmid pH5043 (19). PCR products from one 100-μl reaction were used to transform yeast strain CY114. His+ transformants were screened by PCR to confirm the presence of the arp7ΔHIS3 or arp9ΔHIS3 deletion alleles.

**Ge1 Filtration and Western Analyses**—Crude whole cell extracts were prepared and analyzed by gel filtration on a Superose 6 gel filtration column as described previously (21). Superose 6 fractions (0.5 ml) were trichloroacetic acid-precipitated, resuspended in SDS sample buffer, separated on 10% SDS-PAGE gels, and transferred to nitrocellulose. Immunoblots were probed with either the 12CA5 monoclonal antibody (Babco, Emeryville, CA) to detect SW12 (21) or with a rabbit polyclonal antibody to RPD3; blots were developed with a chemiluminescent substrate as described (22).

**RESULTS AND DISCUSSION**

**MALDI-TOF Mass Spectrometry Analysis**—Purification of the yeast SWI/SNF complex through four chromatographic steps yields a preparation that is >50% pure and is primarily composed of 10 polypeptides (8, 16). A small 11th subunit, SNF11, is not visualized by silver staining (23). Previous studies have identified the genes that encode 8 of the 11 SWI/SNF subunits (8, 23–25). The genes that encode the SWP82/p78, SWP61/p50, and SWP59/p47 subunits have not been identified to date. To identify genes encoding the SWP59/p47 and SWP61/p50 subunits, a SWI/SNF preparation was separated by SDS-PAGE and the protein bands corresponding to SWP73 (a positive control), SWP61/p50, and SWP59/p47 were each excised and digested in situ with trypsin. The molecular masses of the tryptic peptides obtained from each protein band were measured by MALDI-TOF mass spectrometry. The resulting peptide masses were used in a search of the yeast data base to identify proteins in the band of interest (26, 27).

Fig. 1 shows an example of mass spectrometric peptide mapping for the SWP61/p50 subunit. The measured molecular masses of the peptides were used in a search of the S. cerevisiae yeast data base. Twenty peptides were found to match the calculated molecular masses of theoretical peptides from the ARP7 gene product (GenBank™ accession number 809599) with mass accuracy better than 0.2 dalton (accounting for 52% of the protein sequence (18)). Likewise, MALDI-TOF analysis of peptides from the SWP73 subunit uniquely identified the SWP73 gene. The predicted ARP7 protein has a molecular mass of 53.81 kDa, which is consistent with the size of the SWP61/p50 subunit. The protein identifications were further verified by fragmentation of the tryptic peptides in a liquid chromatography tandem mass spectrometry experiment using a LCQ mass spectrometer (Finnigan Corp., San Jose, CA) and the search routine PepFrag (28) (data not shown). Thus, the SWP61/p50 subunit appears to be encoded by the ARP7 gene, which encodes an actin-related protein (3).

In contrast to the unique identification of the gene encoding the SWP61/p50 subunit, MALDI-TOF analysis of the SWP59/p47 subunit yielded two different genes. Sixteen tryptic peptides from the SWP59/p47 band matched the calculated molecular masses of predicted peptides from the RPD3 gene product (accounting for 53% of the protein sequence), whereas 22 tryptic peptides matched the predicted peptides from the ARP9 gene product (accounting for 60% of the protein sequence; GenBank™ accession number 798959). Both sets of identifications were accurate to better than 0.2 dalton. The predicted molecular masses of ARP9 (53.074 kDa) and RPD3 (48.904 kDa) are both consistent with the size of the SWP59/p47 subunit. The ARP9 gene, like ARP7, encodes an actin-related protein (3), whereas the RPD3 gene encodes a histone deacetylase (29, 30).

**RPD3 Is a Subunit of a Distinct 2000-kDa Complex**—Strains that harbor mutations in SWI/SNF subunit genes exhibit a battery of characteristic phenotypes; swi/snf mutants grow...
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slowly on media that contain glucose, and they cannot grow on media that contain galactose, glycerol, or sucrose as sole carbon sources. Furthermore, swi/snf mutants show defects in transcription of several genes (e.g. HO), and several activators, such as GAL4, require SWI/SNF function for full activity. A deletion of the RPD3 gene does not result in a defect in HO expression or in the loss of transcriptional enhancement by GAL4. In fact, a deletion of RPD3 causes a large increase in HO expression (31), and a rpd3 deletion also suppresses the defect in HO transcription due to inactivation of SWI/SNF (32). Strains containing rpd3 mutations also do not show the growth defects that are characteristic of mutations in SWI/SNF subunit genes. These genetic studies suggest that it is unlikely that RPD3 encodes a subunit of the SWI/SNF complex. Previous studies, however, have shown that RPD3 is a subunit of a 2000-kDa protein complex that also contains the SIN3 protein (33). One possibility is that this RPD3 complex is equivalent to the 2000-kDa SWI/SNF complex. Alternatively, the RPD3 complex may be distinct from SWI/SNF, but both complexes may co-purify in our purification scheme. To test these possibilities, we used a gel filtration assay to determine whether disassembly of one complex altered the integrity of the second complex.

Extracts were prepared from wild-type, sin3Δ, or swi1Δ swi2Δ swi3Δ strains and fractionated by gel filtration. Fractions were assayed by immunoblotting using antibodies to RPD3 or the SWI2/SNF2 subunit of SWI/SNF. When extracts were prepared from a wild-type strain, both RPD3 and SWI2 elute at an apparent molecular mass of 2000 kDa (Fig. 2 and data not shown (21); peak elution in fraction 20). However, when extracts were prepared from a sin3Δ strain, the elution of RPD3 shifted to a much smaller apparent molecular mass (~670 kDa; fraction 25), whereas the elution of SWI2/SNF2 remained unchanged (Fig. 2). In contrast, the elution of RPD3 was not changed when extracts were prepared from the swi1 swi2 swi3 triple mutant (Fig. 2), which causes disassembly of SWI/SNF (Ref. 21 and data not shown). These data indicate that RPD3 is not a subunit of SWI/SNF, but that the large RPD3 complex is a distinct complex that co-purifies with SWI/SNF. Consistent with this view, the RPD3 complex binds to DNA cellulose and to Ni2+ resins even in the absence of a hexahistidine tag, and the SWI/SNF complex purified from a sin3Δ strain lacks detectable RPD3 (data not shown).

ARP7 and ARP9 Are Required for SWI/SNF Function—To address the functional role of ARP7 and ARP9 in SWI/SNF function, strains harboring deletion alleles of either ARP7 or ARP9 were constructed and analyzed for growth and transcriptional defects. Like other swi/snf mutants, arp7 and arp9 mutants grow slowly on glucose media, and they are unable to form single colonies on media that contains glycerol or sucrose as the sole carbon source (Fig. 3). The arp9 mutant was also unable to grow on galactose media, whereas the arp7 mutant had a slow growth phenotype on galactose (Fig. 3). The strong growth defect on sucrose media is consistent with a defect in expression of the SWI/SNF-dependent gene, SUC2.

In addition to the growth properties of arp7 and arp9 mutants, we analyzed expression of a SWI/SNF-dependent gene, HO, and the functioning of the GAL4 activator. Disruption of the SWI/SNF complex leads to a 10–100-fold decrease in expression of a chromosomal HO-lacZ fusion gene (22) and an 8–15-fold reduction in the ability of the GAL4 activator to enhance transcription from two low affinity, nucleosomal GAL4 binding sites (22). Deletion of ARP7 or ARP9 results in a 7–10-fold decrease in expression of the HO-lacZ fusion (Fig. 4A). Likewise, deletion of ARP7 or ARP9 led to a 5–11-fold reduction in expression from the GAL4-dependent reporter gene (Fig. 4B). Thus, the phenotypes of arp7 and arp9 mutants are very similar to those of known swi/snf mutants, and the genetics are fully consistent with the MALDI-TOF identification of ARP7 and ARP9 encoding SWP61/p50 and SWP59/p47.

Fig. 2. RPD3 is a subunit of a large complex distinct from SWI/SNF. Whole cell extracts from wild-type (CY114), sin3Δ (CY35), or swi1Δ swi2Δ swi3Δ (CY93) strains were fractionated on a fast protein liquid chromatography Superose 6 gel filtration column, and fractions were assayed for SWI2 or RPD3 by immunoblot. Peak elution of SWI2 or RPD3 is marked with a dot above the corresponding fraction. Arrows denote the elution of the calibration protein, thyroglobulin (669 kDa), and position of the void volume (~7000 kDa).

Fig. 3. Deletions of ARP7 or ARP9 cause growth defects similar to swi/snf mutants. Isogenic strains harboring deletions of either ARP7 (Cy745), ARP9 (Cy744), or SWI2/SNF2 (CY407) were streaked on plates containing the indicated carbon sources and incubated for 3–5 days at 30 °C.
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Fig. 4. ARP7 and ARP9 are required for expression of an HO-lacZ fusion gene and for full functioning of the GAL4 activator.

A, HO-lacZ expression. Isogenic wild-type (CY114), arp7Δ (Cy745), or arp9Δ (Cy744) strains were grown to mid-log phase in YEP medium containing 2% glucose and then analyzed for β-galactosidase activity. Miller units were normalized to percentages of wild-type levels. Analyses were performed in triplicate, and the values were averaged; values varied by <20%. B, GAL4 reporter. A 2-kb GAL4 reporter plasmid containing 2% glucose and then analyzed for

in vivo

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members of the actin superfamily have been maintained during evolution as subunits of the SWI/SNF complex.

The actin ATPase domain is structurally identical to the ATPase domain of the HSP70 family of chaperones, which includes HSC70 and bacterial DnaK. A hallmark of this family of ATPases is that binding of ATP and the subsequent, slow hydrolysis of ATP (0.02 min−1 for DnaK (34)) are linked to large protein conformational changes (discussed in Ref. 35). The presence of two actin-related proteins in the yeast SWI/SNF complex suggests that the complex may contain a total of three ATP binding subunits (SWI2/SNF2, ARP7, ARP9); each is required for SWI/SNF function in vivo. What roles do each of these ATP binding proteins play in SWI/SNF function? SWI2/SNF2 is likely to generate most of the mechanical energy needed for chromatin remodeling, because it can hydrolyze about 1000 ATP molecules/min in the presence of nucleosomal DNA (8). In contrast, ARP7 and ARP9 may undergo only a few rounds of ATP binding and hydrolysis that control structural rearrangements within the SWI/SNF complex. Changes in SWI/SNF conformations may be required for interactions with nucleosomal components (i.e., the histones) or for chromatin remodeling activity. Purification of mutant SWI/SNF complexes that contain ATPase-defective versions of either ARP7 or ARP9 will be invaluable for defining their roles in ATP-dependent nucleosome remodeling.

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